Chemical Physics 442 (2014) 77–80

Contents lists available at ScienceDirect

Chemical Physics

journal homepage: www.elsevier.com/locate/chemphys

On the photostability of the disulfide bond: An electronic or a structural property?

Anne B. Stephansen, Martin A.B. Larsen, Liv. B. Klein, Theis I. Sølling *

Department of Chemistry, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen Ø, Denmark

ARTICLE INFO

Article history:
Available online 24 February 2014

Keywords:
Disulfide
Photostability
Photodissociation
Time-resolved mass spectrometry
CASSCF

ABSTRACT

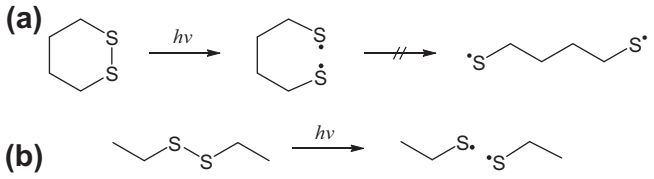
Photostability is an essential property of molecular building blocks of nature. Disulfides are central in the structure determination of proteins, which is in striking contradiction to the result that the S–S bond is a photochemically labile structural entity that cleaves to form free radicals upon light exposure. In an earlier contribution we hypothesized that the key to the photostability of some disulfides may be found in a cyclic structural arrangement. Here we provide further evidence to support this hypothesis by showing that straight chain disulfides undergo ultrafast S–S dissociation on a sub 50 fs timescale without further ado. In a cyclic motif resembling the cysteine–disulfide bond in proteins, light can perturb the S–S bond to generate short-lived diradicaloid species, but the sulfur atoms are conformationally restricted by the ring that prevents the sulfur atoms from flying apart. Conversely, in a straight chain conformation, light perturbation results in two separated RS· radicals because there is no restoring force to counteract the repulsive motion of the sulfur atoms. For the cyclic conformation this restoring force is provided by the cyclic framework, and thus the photostability of disulfide-bonds must be ascribed a cyclic structural arrangement.

Disulfide bonds are key in several systems in nature with examples ranging from the atmospherically relevant sulfur cycle [1] to protein folding and structure determination in terms of the cysteine disulfide-bond [2]. The significance of the latter seems surprising considering that disulfides also are known to be cleaved upon exposure to heat and UV-light and form free radicals that can react in various chemical processes [3,4]. This contradicts the role as a central building block in proteins where photostability is pivotal. Recently, we put this opposing issue into question by investigating the sulfur–sulfur photolysis of the cyclic, aliphatic disulfide, 1,2-dithiane (Scheme 1a), whose cyclic structure mimics the structural motif of the cysteine-linking disulfide-bond in proteins [5]. We found that in the case of 1,2-dithiane, the disulfide-bond does indeed break on the excited state on a sub 100 femtosecond (fs) timescale, but only to result in a diradical where the –S· ends oscillate around an excited state minimum resembling a folded diradical. The involved wiggling motion couples the S_1 state to the ground state surface through a conical intersection, and the end result is ultrafast repopulation of the ground state. The dynamics of the system thus ensures that the formed radicals will stay in close proximity during the relaxation process so that the final result of light exposure is ground state 1,2-dithiane molecules with an intact S–S bond. We hypothesized that the key to the photostability of the disulfide-bond of 1,2-dithiane is built into the confined cyclic arrangement of these structures, and that this confinement also is responsible for the photostability of tertiary structure of proteins.

The importance of photostability is a ubiquitous and fundamental necessity for natural systems. For the DNA bases the mechanism that ensures photostability is similar to the mechanism exhibited by 1,2-dithiane, namely that the involved electronic states couple along the degrees of freedom that become activated in the absorption process which facilitates ultrafast repopulation of the ground state [6–8]. In a broader biological perspective, correlations of protein structure vs. spectroscopic properties of proteins show that fluorescence from excited tryptophan-moieties is effectively quenched by disulfide-bonds linking nearby cysteine-moieties [9–12]. However, if the sulfur–sulfur bond is reduced to form thiols the fluorescence quantum yield increases drastically. Already long before the quenching mechanism was established, it was suggested that disulfides could be considered as "energy sinks" due to the efficient loss of fluorescence via an intramolecular non-radiative process [13]. Later it was shown that the fluorescence quenching is due to electron transfer from tryptophan towards the disulfide[10] and the quenching process can be

* Corresponding author. Present address: Maersk Oil Research and Technology Centre, TECH 2 Building, Level 1, Unit 107 Al Gharrafa Street, Al Rayyan, Education City, PO Box 210112, Doha, Qatar.
E-mail address: theis@kiku.dk (T.I. Sølling).

http://dx.doi.org/10.1016/j.chemphys.2014.02.005
0301-0104/



Scheme 1. Photolytical S–S bond cleavage in (a) 1,2-dithiane, and (b) diethyl disulfide.

observed in most disulfide-containing proteins. It has been noted that tryptophan-moieties situated in the vicinity of disulfides is a common structural motif in proteins. However, the biological implication of this design is still unknown [14]. It is relevant to establish the role of the sulfur–sulfur bond in such a structural motif to prove whether the S–S bond protects the rest of the protein from the potentially harmful fluorescence from the tryptophan. In order for the disulfide to act as an efficient UV-shield, photostability is crucial.

The focus of this contribution is to test whether the photostability observed for 1,2-dihiane is an electronic sulfur–sulfur bond property or a structural property ascribed to the cyclic framework. We address this issue by investigating the photoinduced dynamics of the linear counterpart, diethyl disulfide (Scheme 1b). In order to allow for direct comparison with our earlier results we have used the same experimental and theoretical methods involving femtosecond time-resolved mass spectrometry (TRMS) and Complete Active Space Self Consistent Field calculations. The photochemistry of straight chain alkyl disulfides is already fairly well-studied but the reported fragmentation mechanisms are ambiguous. Dimethyl disulfide constitutes the foremost studied aliphatic disulfide and two competing primary photodissociation channels have been suggested [15–20].

$$CH_3SSCH_3 + h\nu \rightarrow 2CH_3S^{\cdot} \quad (1)$$

$$CH_3SSCH_3 + h\nu \rightarrow CH_3SS^{\cdot} + CH_3^{\cdot} \quad (2)$$

Using time-resolved transient absorption measurements Kumar et al. [16] suggested that both channels are in play following resonant ($\sigma^*_{ss} \leftarrow n_s$) electronic excitation (248 nm), whereas Lee et al. [15] used gas phase photofragment translational energy and angular distribution spectroscopy to show that photodissociation induced by 248 nm light exclusively follows channel (1) in a fast and bond-specific process, while irradiation with shorter wavelengths results in a competition of the two fragmentation channels. The S–S reaction coordinate is of main interest here, but due to the ambiguity of earlier investigations we also considered the C–S reaction coordinate.

The femtosecond TRMS experiments were conducted on a molecular beam generated in a supersonic expansion into vacuum. Diethyl disulfide was excited by a 267 nm pump pulse, corresponding to the red edge of the S_1 absorption band, and subsequently two 400 nm probe pulses ionized the evolving excited state population (ionization energy ≈ 8.27 eV [21]). The resulting ion current was measured as a function of pump–probe delay. The experiments were assisted by a series of *ab initio* calculations. Diethyl disulfide has many local minimum energy structures on the ground state potential energy surface. We chose to focus on the conformation shown in Fig. 1 denoted GGG specifying the structural orientation of the C–S–S–C segment (*gauche*). This geometry is both the most stable conformer and the one that most closely resembles the disulfide motif seen in proteins [2,22], yet, still in a non-cyclic arrangement. Frequency calculations verified that this geometry represents a minimum energy structure (no imaginary frequencies). To test whether other conformations also present at biological temperature exhibit similar properties we also investigated the TGT (*trans*) conformer (S1). G3MP2 [23] calculations predicted the energy difference between the GGG and TGT conformers to be 2.5 kJ/mol, i.e. considering the accuracy of the method [23,24], these structures are essentially equal in

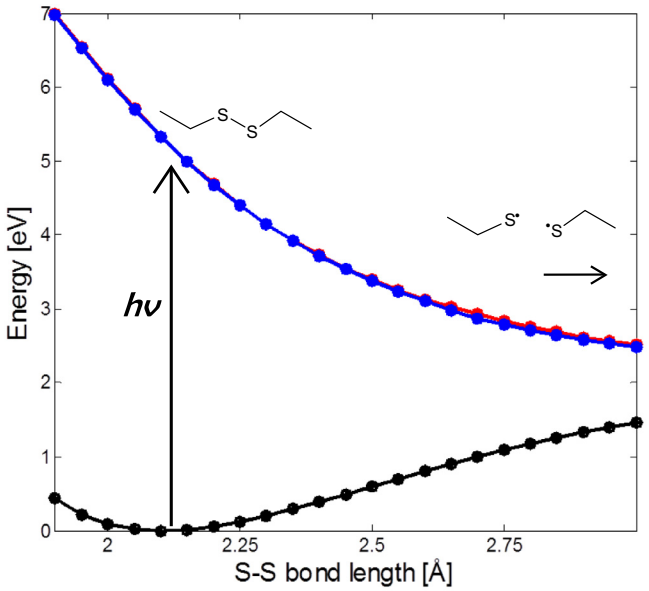


Fig. 2. Potential energy surface of the three lowest singlet excited states of diethyl disulfide calculated for the GGG conformer along the S–S reaction coordinate at the B3LYP/6-31+G(2df,p)//CASSCF(10,8)/6-31+G(2df,p) level of theory.

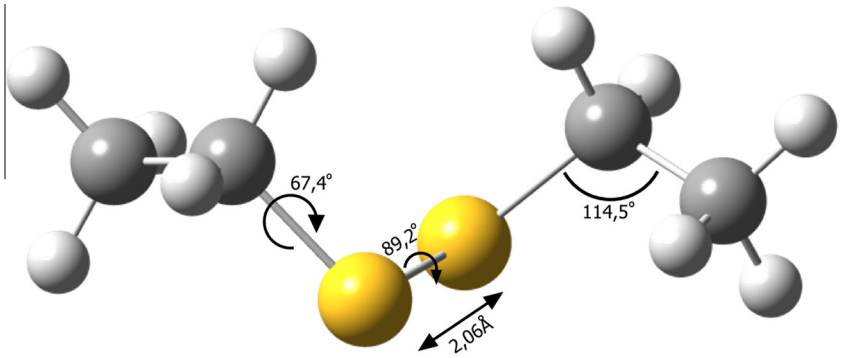


Fig. 1. Optimized geometries (B3LYP/6-31+G(2df,p)) of the GGG conformer of diethyl disulfide with selected bond lengths and angles as indicated.